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Oxidative and nitrosative signalling in pulmonary arterial hypertension — Implications for development of novel therapies

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ABSTRACT

Pulmonary arterial hypertension (PAH) is a syndrome characterised by an increase in pulmonary vascular resistance. This results in elevated resting pulmonary artery pressure and leads to progressive right ventricular (RV) failure, secondary to increased afterload. Although initially thought to be a disease driven primarily by endothelial dysfunction with a resultant vasoconstrictor versus vasodilator imbalance, it has become increasingly apparent that the rise in pulmonary vascular resistance that causes RV failure is also attributable to pulmonary vascular remodelling. This inflammatory, hyper-proliferative and anti-apoptotic phenotype is accompanied by a metabolic switch from physiological mitochondrial oxidative phosphorylation to aerobic glycolysis. The molecular pathways triggering this cellular metabolic shift have been the subject of extensive investigation, as their discovery will inevitably lead to new therapeutic targets. Reactive oxygen/nitrogen species (ROS/RNS) including hydrogen peroxide, superoxide and peroxynitrite are second messenger molecules that are involved in functional oxidative and nitrosative modification of proteins. Dysregulation of oxidative signalling caused by an excess of ROS and RNS relative to antioxidants has been heavily implicated in the underlying pathophysiology of PAH and likely participates in this metabolic reprogramming. This review will focus on the role of oxidative signalling and redox reactions to the molecular pathology of PAH. In addition, promising novel therapeutic agents targeting these pathways will be discussed.

1. Introduction

Pulmonary hypertension (PH) is a chronic disease of small pulmonary vessels characterised by progressive remodelling of the pulmonary vasculature, resulting in increased pulmonary vascular resistance (PVR), right heart failure and death.

The World Health Organization (WHO) categorises the different aetiologies of PH into groups sharing similar pathophysiology and haemodynamics (Simonneau et al., 2013; Galie et al., 2016). WHO group 1 PH, pulmonary arterial hypertension (PAH), is characterised by pre-capillary pulmonary resistance (McGoon & Kane, 2009) defined by pulmonary artery wedge pressure (PAWP) ≤ 15 mm Hg and PVR > 3 Wood units (WU) in the absence of secondary causes of precapillary PH (Galie et al., 2016). This is in contrast to the other 4 groups, where secondary causes of elevated pulmonary pressures occur due to underlying pathology originating outside of the pulmonary arterial system. Although therapeutic options for PAH are improving, it remains a progressive disease with high mortality, often affecting young patients.

The last decade has witnessed a paradigm shift in our understanding of the pathophysiology of PAH. Rather than simply a vasoconstrictor/

Abbreviations: ACE, angiotensin converting enzyme; ADMA, asymmetric dimethyl-L-arginine; AngII, angiotensin II; BH_2, dihydrobiopterin; BH_4, tetrahydrobiopterin; BMPR-II, bone morphogenetic protein receptor type II; Cav-1, caveolin-1; cGMP, cyclic guanosine monophosphate; CypA, cyclophilin A; ERA, endothelin receptor antagonist; ET1, endothelin-1; eNOS, endothelial nitric oxide synthase; HIF-1α, hypoxia-inducible factor-1α; H_2O_2, hydrogen peroxide; Na^+K^+ pump, sodium potassium ATPase pump; mTOR, mammalian target of rapamycin; MCT, monocrotaline; NO, nitric oxide; NOX, NADPH oxidase; O_2^-, superoxide; OH, hydroxyl radical; $ONOO^-$, peroxynitrite; PDE-5, phosphodiesterase-5; PDGF, platelet derived growth factor; PH, pulmonary hypertension; PAH, pulmonary arterial hypertension; PAP, pulmonary artery pressure; PDH, pyruvate dehydrogenase; PDK, pyruvate dehydrogenase; PVR, pulmonary vascular resistance; RV, right ventricle; ROCK, Rho kinases; ROS, reactive oxygen species; RNS, reactive nitrogen species; SERCA, sarco-endoplasmic reticulum calcium ATPase; sGC, soluble guanylate cyclase; SMC, smooth muscle cell; SOD, superoxide dismutase; SrcFKs, Src Family of Kinases; STAT3, signal transducer and activator of transcription 3; TGF-β, transforming growth factor-β; XO, xanthine oxidase.

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vasodilator imbalance, this condition is underpinned by multiple molecular, genetic and epigenetic abnormalities, which cause endothelial dysfunction, pathological vascular remodelling and mitochondrial metabolic abnormalities (Ryan & Archer, 2015). Existing pharmacotherapies, whilst targeting multiple aspects of underlying pathogenesis, have not demonstrated the capacity to adequately reverse the underlying disease process, and only confer modest survival benefits (Humbert et al., 2010; Morrell et al., 2013). Therefore, the discovery of molecular signalling pathways and culprit genetic abnormalities underlying PAH is opening avenues for novel therapeutic interventions to help combat this deadly disease.

In comparison to the systemic vascular bed, the normal pulmonary vasculature is a high-flow low-pressure system. In PAH, structural alterations cause progressive vascular remodelling of all three vessel layers, resulting in a switch to a pathological high-resistance system. Briefly, concentric plexiform lesions develop in the intimal layer (Tuder et al., 2007), with dysregulated endothelial cell proliferation and aberrant vascular channels forming during the advanced stages of the disease. Extensive medial smooth muscle cell (SMC) hypertrophy and/or hyperplasia (Stenmark et al., 2009) and fibroblast transdifferentiation cause the hallmark neo-muscularisation of previously non-muscular arteries (Jiang et al., 2006). In addition, increased collagen and inflammatory cells in the adventitial layer contribute to pulmonary vascular stiffening (Chelladurai et al., 2012). For a comprehensive background of the histo-pathological changes observed in PAH, the reader is referred to the review by Tuder et al. (2007).

2. Coordination of reactive oxygen & nitrogen species (ROS/RNS), & their signalling in vascular physiology

ROS are formed as products of oxidation–reduction reactions and include free radical molecules — atoms or molecules with a single unpaired electron including superoxide (O_2^-), hydroxyl radical (OH^-) and lipid peroxy – radical (LOO) – and non-free radical species like hydrogen peroxide (H_2O_2). RNS like ($ONOO^-$) are formed by the reaction between nitric oxide (NO) and O_2^- (Beckman et al., 1990). Cellular ROS are generated during regulated physiological processes predominantly as by-products of mitochondrial respiration, as well as NADPH oxidase activity at the membrane, "uncoupled" endothelial nitric oxide synthase (eNOS) (Chen et al., 2010) and xanthine oxidase (Elahi et al., 2009).

Coordinated and compartmentalised ROS and NO production play an essential role in regulating vascular tone, as well as more chronic signalling pathways driving inflammation and hypertrophy. Akin to phosphorylation, receptor-coupled ROS production drives reversible oxidative post-translational modification of susceptible proteins, in addition to irreversible modifications (Hawkins et al., 2009). Glutathionylation is one example of a stable, yet enzymatically reversible reaction that adds a negatively charged 305 Da glutathione adduct to protein cysteine residues with well-documented steric effects and subsequent change in the function in key cellular proteins (Dalle-Donne et al., 2008; Ho et al., 2013). An increasing number of cellular enzymes and membrane channels/pumps are recognised to be tightly regulated by ROS (Table 1), including the sodium potassium ATPase channel (Na^+–K^+ pump), and sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) which have both been shown to mediate altered systemic arterial tone (Schoneich & Sharov, 2006; Liu et al., 2013).

Although ROS directly impair the function of susceptible proteins via oxidative post-translational modifications such as glutathionylation, this is normally minimised by cellular antioxidant enzymes such as catalase, peroxiredoxins, glutaredoxin and glutathione peroxidases (Ho et al., 2013).

In the healthy vasculature, endothelial NO synthase (eNOS) oxidises L-arginine to L-citrulline and NO, with associated transfer of electrons from the reductase to oxidase domain (Zweier et al., 2011). NO secondarily mediates its vasodilator effects via the soluble guanylate cyclase-cyclic guanosine monophosphate-protein kinase G (sGC-cGMP-PKG) signalling cascade (Arnold et al., 1977) which also contributes to its longer-term vascular benefits including inhibiting vascular SMC proliferation and platelet aggregation (Albrecht et al., 2003). However, under conditions of substrate/co-factor depletion, or higher local ROS levels, eNOS can become uncoupled, resulting in predominant production of O_2^- (Chen et al., 2010; Zweier et al., 2011; Crabtree et al., 2013; Galougahi et al., 2014). This molecular event is a key switch with broad relevance for vascular physiology and pathophysiology.

The reaction between O_2^- and NO results in the production of $ONOO^-$, a key cellular RNS (Chen et al., 2014). $ONOO^-$ oxidises protein thiols forming disulphide, and causes nitration of tyrosyl groups in proteins, forming nitrated proteins, which also contribute to loss or gain of function (Kalyanaraman, 2013).

The balance of ROS/RNS production and cellular protective mechanisms is critical for vascular health. Dysregulation of ROS signalling, and the subsequent oxidation of cellular protein, lipid and DNA (Tabima et al., 2012) is a major molecular switch initiating and perpetuating vascular disease states. The following section will describe the pathogenesis of PAH, and the evidence supporting a role for ROS/RNS in its cause, specifically with regard to dysfunction of the major ROS-generators and perturbation of known signalling pathways.

Table 1
Modulation of proteins by S-glutathionylation. The evidence supporting a role for S-glutathionylation predominantly derives from the systemic circulation, although a role for this in the pulmonary circulation is yet to be explored.
Ho et al. (2013) and Pastore and Piemonte (2013)).

Protein	Modified cysteine	S-glutathionylation and vascular response	Ref
eNOS	Cys689, Cys908	S-glutathionylation of eNOS decreases its activity and increases superoxide production, causing decreased NO-induced vasodilation. In addition, AngII-mediated S-glutathionylation of eNOS results in eNOS uncoupling, reduced activity and results in NADPH-dependent superoxide production and endothelial dysfunction	Chen et al., 2010, Galougahi et al., 2014 and Karimi Galougahi et al., 2016
Na^+K^+ pump	Cys45	NADPH-mediated S-glutathionylation of β-1 subunit of Na^+K^+ pump implicated in increased vascular tone and cardiac contractility	Figtree et al., 2009 and Liu et al., 2012, 2013
SERCA	Cys674	$ONOO^-$ mediated S-glutathionylation results in cGMP-independent vasodilation	Adachi, Weisbrod, et al., 2004
Aldose reductase	Cys298	Aldose reductase mediates TNFα-induced smooth muscle hypertrophy and is inactivated by S-glutathionylation.	Ramana et al., 2007 and Cappiello et al., 2001
P21Ras	Cys118	AngII-induced hypertrophy of vascular SMCs occurs by S-glutathionylation of P21Ras with subsequent activation of the ERK signalling pathway.	Adachi, Pimentel, et al., 2004
K_{ATP} channels	Cys176	S-glutathionylation of Kir6.1/Sur2B channel after exposure to H_2O_2, resulted in reduced vascular endothelium-dependent relaxation	Yang et al., 2011
Sirtuin1	Cys67	Glutaredoxin-2 dependent S-glutathionylation of Sirtuin-1 is essential for normal development of vascular network	Brautigam et al., 2013 and Zee et al., 2010

Legend: AngII — angiotensin II, cGMP — cyclic guanosine monophosphate, Cys — cysteine, eNOS — endothelial nitric oxide synthase, H_2O_2 — hydrogen peroxide, K_{ATP} — ATP-activated potassium channels, NADPH — nicotinamide adenine dinucleotide phosphate, $ONOO^-$ — peroxynitrite, and SERCA — sarco/endoplasmic reticulum calcium ATPase.